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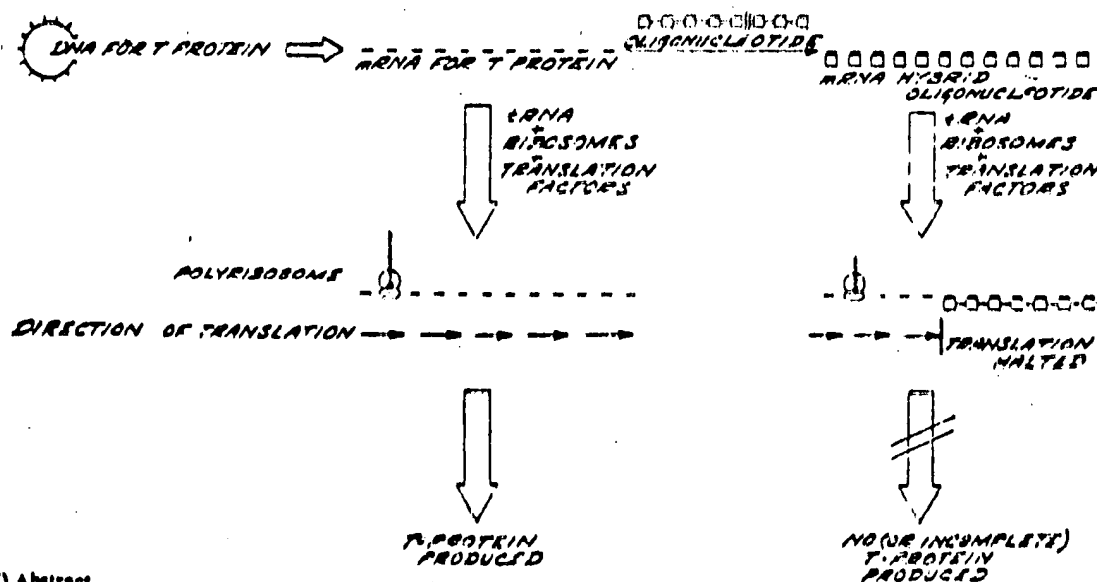
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(54) Title: OLIGONUCLEOTIDE THERAPEUTIC AGENT AND METHODS OF MAKING SAME



(57) Abstract

For use in controlling biologic functions in an organism, a stabilized oligonucleotide, preferably in a phosphotriester form, having a base sequence substantially complementary to a portion of messenger ribonucleic acid coding for a biological component, such as a protein, of the organism. The oligonucleotide has about fourteen bases or more, such as twenty-three bases, and can be a deoxyribonucleotide. The oligonucleotide sequence can be derived from the organism's ribonucleic or deoxyribonucleic acid that codes for a vital protein, and can be synthesized in bulk either chemically or by insertion into a plasmid.

OLIGONUCLEOTIDE THERAPEUTIC AGENT
AND METHODS OF MAKING SAME

BACKGROUND OF THE INVENTION

5 This invention relates generally to controlling biologic functions, such as for antibiotic purposes, and more particularly, to utilizing hybridization techniques of the type having messenger ribonucleic acid attached to oligonucleotides for in vivo protein synthesis regulation.

0 In the field of pharmacology, the use of therapeutic agents has long been recognized as an effective way to control diseases. Such agents are often used in treating bacterial or viral infections, chemical imbalances and the like, to cure, or at least mitigate, the diseased state. Although researchers occasionally discover new therapeutic agents after major breakthroughs have elucidated the molecular basis of a disease, more often they must rely on observing for antibiosis or modifying the chemical structures of functionally related chemicals.

With respect to antibiotic agents, some are quite effective at the outset, but over time many organisms become resistant or totally immune to their action. Additionally, very few effective anti-viral agents have ever been developed, and without explicit, detailed knowledge of an infecting organism's physiology, the development of new operative agents remains haphazard.

Thus, there exists a definite need for a methodology enabling the systematic design of new antibiotics and other therapeutic agents that is versatile and inexpensive, yet produces agents that are both extremely specific and effective. The present invention fulfills these needs.



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SUMMARY OF THE INVENTION

The present invention provides a methodology of identifying and constructing therapeutic and other agents for use in living organisms that substantially reduces the uncertainty surrounding the development of new antagonists, significantly increasing the scope of materia medica. Moreover, the agent construction of the present invention lends itself readily to simple manufacture, even in large quantities, is extremely effective in use, and attains its improved results without undue cross-reactions.

In a presently preferred embodiment of the invention, by way of example and not necessarily by way of limitation, a stabilized oligonucleotide, preferably in a phosphotriester form, is provided having a base sequence substantially complementary to a portion of messenger ribonucleic acid coding for a biological component of an organism. Due to the complementary nature of the oligonucleotide and the messenger ribonucleic acid, the two components can readily hybridize under appropriate conditions to control synthesis of the organism's biological component and, if the protein is vital to the organism's viability, to act as an antibiotic.

A method, in accordance with the present invention, of developing therapeutic agents may typically include the steps of: providing a base sequence of an organism's nucleic acid that contains at least a portion of the genetic information for a biological component of the organism, and synthesizing an oligonucleotide the sequence of which is derived from the base sequence, for



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subsequent hybridization with the messenger rib nucleic acid specific for the biological component. The biological component may be a vital protein, or simply a hormone such as the gonadotropin, follicle stimulating hormone. The order of the base sequence may be determined from deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), preferably messenger ribonucleic acid (mRNA). Alternatively, the desired oligonucleotide base sequence may be determined from the biological component's sequence, as when the biological component is a protein. The preferred oligonucleotide has a minimum of about fourteen or more bases, such as about twenty-three bases, and for increased stability, may be transformed to a more stable form, such as a phosphotriester form, to inhibit degradation during use.

To produce large quantities of the oligonucleotide, it may be synthesized chemically, such as in automated machines, or inserted into a plasmid, such as pRR322, for cloning. The plasmid insertion may be accomplished with linker base sequences, such as GATTC-GAATC or CTAAGCTTAG, which are susceptible to degradation by Hind III restrictive nuclease or Alu I restriction nuclease. When the order of the base sequence has not been determined, the base sequence can be cloned and then cross-hybridized against messenger ribonucleic acid from the other sources to remove base sequences non-specific to the target.

Another aspect of the present invention is a method of selectively controlling activity of one or more biological components in a cell without substantially interfering with the activity of other biological components in the cell. The method includes the steps of



forming an oligonucleotide having a base sequence substantially complementary to a portion of mRNA coding for the specific biological component, and introducing the oligonucleotide into the cell for hybridization with the selected mRNA. This causes blocking of the translation of the mRNA into protein. The oligonucleotide may have at least about fourteen bases or more, such as about twenty-three bases. The target mRNA can code for a protein, such as the hormone, follicle stimulating hormone. This hormone has an alpha and beta chain, and the oligonucleotide should be specific for the mRNA coding for the beta chain to avoid cross-reacting with other gonadatropin mRNA. A suitable oligonucleotide base sequence would be ACCACGCGR₁CCR₂ATGACGATGTG, wherein R₁ is G or T and R₂ is also G or T.

In accordance with another aspect of the present invention, a method is provided for inhibiting the infection of a host organism by a foreign organism. This method entails isolating a base sequence containing at least a portion of the genetic information coding for a vital protein from the foreign organism's nucleic acid; synthesizing an oligonucleotide, the order of which is derived from the base sequence and substantially complementary to the messenger ribonucleic acid coding for the protein; and treating the foreign organism with an effective amount of the oligonucleotide to hybridize with a portion of the messenger ribonucleic acid and block translation of the protein. The oligonucleotide, which can be a deoxyribonucleotide, can be transformed to a more stable form, such as a phosphotriester form, to inhibit degradation, and the order of the sequence determined prior to its synthesis. Further, to increase the oligonucleotide's specificity, it may be cross-hybridized



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dized against mRNA from different organisms, such as the host organism, to remove non-unique oligonucleotide sequences.

It will be appreciated from the foregoing that the present invention satisfies a long existing need for improved methods of developing therapeutic agents for use in living organisms, and represents a significant advance over previously available methods, principally because it is very versatile, and yet provides a very specific agent against a biological component. Other aspects and advantages of the invention will become apparent from the following more detailed description taken in conjunction with the accompanying drawings.



BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flow diagram showing the central dogma of molecular biology;

5 FIG. 2 is a flow diagram showing normal translation of messenger ribonucleic acid (mRNA) into T protein, as well as a synthetic oligonucleotide of the present invention blocking translation of the T protein;

FIG.3 is a list of the viral deoxyribonucleic acid (DNA) code specific for SV-40 T protein, and the related mRNA and oligonucleotide;

10 FIG.4 is a flow diagram showing the construction of high yield plasmids containing the T protein oligonucleotide;

15 FIG.5 is a flow diagram showing the use of restriction nucleases to cleave the plasmids to give a purified T protein oligonucleotide;

FIG. 6 is a flow diagram showing the treatment of a DNA sequence to form DNA polyphosphotriesters; and

20 FIG. 7 is a chart showing the partial amino acid sequence of follicle stimulating hormone, as well as the predicted mRNA sequence and related oligonucleotide family.

DETAILED DESCRIPTION OF THE INVENTION

Referring now to the drawings, and particularly to FIGS. 1 and 2, there is shown the so-called "central dogma" of the molecular biology of life. Basically, it is now accepted that deoxyribonucleic acid (DNA) carries the genetic code for almost all living organisms. The code exists in the form of an organized sequence of four nucleotide bases attached to a phosphorylated, deoxyribose sugar backbone. Generally, DNA exists in the form of a double strand helix formed of two oppositely directed strands, which are held together in an opposing manner by various weak forces.

A primary constituent of these weak forces are the so-called hydrogen bonds that exist between nucleotides on the opposing strands. The four bases, adenine (A), cytosine (C), guanine (G), and thymine (T), form hydrogen bonds generally in only one fashion: A with T and C with G. Thus, by knowing the sequence of one strand, the sequence of the second strand can be readily determined.

Another aspect of the central dogma is that proteins are produced indirectly from the DNA strand, through messenger ribonucleic acid (mRNA). Apparently, mRNA, which has the same structure as single stranded DNA except with a ribose backbone and with uracil (U) replacing thymine, is transcribed directly from one DNA strand and has an essentially opposite base sequence, i.e., if a DNA strand sequence is 5'...ACGT...3' the transcribed mRNA sequence is 3'...UGCA...5'.

An additional aspect of the central dogma relates to the translation of mRNA into proteins.



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5 Briefly, excluding initiation sites and the like, each
three nucleotide base grouping (triplet code) codes for
one amino acid of a protein. Therefore, by knowing the
mRNA sequence of a protein, its amino acid sequence can
generally be determined. However, the reverse is not
true, that is, knowing the amino acid sequence does not
guarantee precise knowledge of the mRNA sequence.
This stems from the fact that there exist 64 (4^3)
possible triplet codes, yet there exists only about
10 twenty amino acids, allowing some amino acids to have
multiple triplet codes.

15 The similarity in the structure of DNA and mRNA
strands creates interesting effects. Most notably, if
complementary DNA and RNA strands exist contemporaneously
in a solution, under certain established conditions the
strands can anneal, forming hybrids. One important
factor in proper annealing is the melting temperature,
which can be calculated according to Britten et al,
Methods of Enzymology 29:362 (1974).

20 In accordance with the present invention, a
synthetic oligonucleotide having a base sequence capable
of substantially matching that of a chosen mRNA is
provided for hybridization with that mRNA. Once such a
hybrid exists, the translation of the mRNA into protein
25 becomes significantly inhibited. If the inhibited
protein is vital to an organism's survival, the organ-
ism's viability, i.e., either growth or continued life,
is jeopardized. Importantly, the oligonucleotide can be
designed specifically for the mRNA coding for just one
30 protein, and should not cross-react with mRNA for other
proteins.



The method of developing the oligonucleotide entails basically two steps. As described more fully below, a possible first step is to determine the appropriate sequence of the mRNA specific for the protein to be inhibited, and a second step is to manufacture an oligonucleotide complementary to the mRNA. Once made, the oligonucleotide can be treated into a phosphotriester form for increased stability.

A variety of techniques exists for determining nucleic acid base sequences. In many instances the sequence of the mRNA or the gene have been determined and published in the biochemical literature. In fact, researchers have determined the complete nucleotide sequence for the SV-40 virus (Reddy et al, Science 200:494 (1978)). As is well known, an alternative method entails isolating and purifying mRNA in sufficient quantities to permit sequencing studies, but this can prove difficult due to the relative instability and, in some cases, extreme rarity of many mRNA sequences.

Still another method for determining nucleic acid base sequences requires resolving the amino acid sequence from the target protein. After determining the amino acid sequence of the target protein in purified form, a sequential degradation utilizing commercially available protein sequences (e.g. from Beckman Instruments, Fullerton, California) can be used to provide the amino acid sequence. Once this has been obtained, knowledge of the triplet code can be applied to give prospective base sequences. An example of such a process for the hormone glucagon can be found in Tullis et al, Biochemical and Biophysical Research Communications 93:941 (1980).



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Once the sequence of the appropriate nucleic acid and the desired mRNA sequence have been determined, an oligonucleotide, such as deoxyribonucleotide, complementary to the mRNA can be constructed. A number of synthetic techniques are known, most typical is the diester approach described by Agarwal et al, Nature 227: 27 (1970), and oligonucleotide synthesizers may be purchased commercially from Vega Biochemicals, P.O. Box 11648, Tucson, Arizona and Biologicals, Inc., Toronto, Canada.

If the desired oligonucleotide sequence is unknown, a suitable oligonucleotide can be prepared as follows. After isolating mRNA from a target organism, multiple copies are made, preferably in the form of DNA, so-called copy DNA (cDNA). This cDNA is then cross-hybridized against mRNA isolated from other organisms, and any cDNA hybridizing is removed. The remaining cDNA is specific only to the target organism, and can serve as the therapeutic agent.

In order to obtain a high degree of specificity, an oligomer of about fourteen or more residues can be constructed. Although shorter sequences will work, longer sequences provide higher specificity. This can readily be seen mathematically. Whereas a ten unit polymer chosen from four bases can have 4^{10} (1,048,576) random combinations, a 20-unit polymer has 4^{20} random combinations, which equals 1.09×10^{12} (1,090,000,000,000).

In spite of the added difficulty in making oligonucleotides of twenty units in comparison to ten bases, it is warranted because the exponential increase



in complexity reduces undesirable cross-reactivity. It has been estimated that a mammalian cell contains about 2×10^8 nucleotides of RNA complexity or, in other words, approximately 200 million nucleotides of unique sequence mRNA, which is equivalent to about 30,000 mRNA sequences. The probability that one of those sequences contains a randomly chosen 20-unit polymer is approximately one in fifty-five hundred. In comparison, a ten-unit polymer has about a one hundred and ninety to one chance for random cross-reaction.

The present invention is illustrated by, but not limited to, the following examples.

EXAMPLE I

SV-40 virus manufactures a vital protein commonly known as the "T protein" or "T antigen protein". As noted earlier, the complete genetic code for the SV-40 virus has been determined, and it is known that residues 5091 to 5071 on the viral genome code for a portion of the T protein mRNA. The sequence of these residues, the viral T protein mRNA sequence, and the designed T protein specific oligonucleotide are shown in Fig. 3. In this case, the T protein specific oligonucleotide is complementary to the viral T protein mRNA, and identical to the portion of the viral DNA code.

Prior to testing the effectiveness of the T protein specific oligonucleotide in vivo, the oligonucleotide can be mixed with total mRNA from an organism to check for cross-reactivity. If it hybridizes, then a different portion of the viral genome coding for the T protein should be utilized. Otherwise, the oligonucleotide is ready for further testing.



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Further testing requires growth of S-40 virus. For purposes of these experiments, SV-40 virus is grown and titered in African green monkey cells, such as the cell line BSC-1, according to Hopps et al, Journal of Immunology 91:416 (1963). The identity of the virus can be confirmed by the following methods:

- a) checking for tumor production after inoculation of the newborn hamsters with the virus;
- b) neutralization of the virus by anti-SV-40 antiserum; and
- c) reaction of the infected cells with anti-SV-40 T antigen directed antibodies prepared by standard techniques.

The isolation of SV-40 mRNA can be accomplished as follows. Total RNA is first obtained by the guanidine hydrochloric acid extraction procedure using glassware previously baked and treated with diethylpyrocarbonate to remove traces of RNase as taught by Cox et al, Methods in Enzymology 12B:120 (1968). The A+ RNA is isolated on oligo-dTcellulose, which can be obtained from Collaborative Research, Waltham, Massachusetts, or P. L. Biochemicals, Inc., Milwaukee, Wisconsin, utilizing the technique described by Bantle et al, Analytical Biochemistry 72:413 (1976). The RNA fractions are assayed for purity and intactness by electrophoresis according to the method described in Bailey et al, Analytical Biochemistry 70:75 (1976). Also, the RNA can be assayed for translatability in the wheat embryo in vitro system described in Marcus, et al, Methods in Enzymology: 30:749 (1974). The in vitro translation products are monitored on sodium lauryl sulphate 9% polyacrylamide gels as described in Laemmli, Nature 227:680 (1970).



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5 This purified A+ mRNA containing the viral
mRNA sequences can hybridize to the synthetic oligo-
nucleotide at 37°C in 0.5 M sodium phosphate buffer, pH
6.8, containing 0.2% sodium lauryl sulphate. Solutions
10 containing about 1 mg A+ mRNA and synthetic oligonucleo-
tide at a concentration of about 100 ug/ml are heated to
100°C for 1-2 minutes, then cooled to 37°C and allowed
to anneal. The extent of the hybridization reaction as a
function of time may be monitored on a gel filtration
column.

15 Actually, while any theoretically suitable
temperature may be used for the hybrid formation, tempera-
tures ranging from 0°C to about 80°C provide for good
hybridization, but preferred temperatures range from
about 10°C to about 40°C. Generally, the optimal
annealing temperature for the formation of specific
20 hybrids is believed to be about 20°C to 25°C below
their melting temperature. Synthetic oligonucleotides
operating at 37.5°C should thus be designed on the
basis of their base sequence and length, such that the
melting temperature is between about 57°C and 62°C
when tested under approximately physiological conditions.

25 For hybridization testing the ratio of the
synthetic oligonucleotide to its mRNA complement is
generally about 30:1. Lower ratios are acceptable,
however, sequences below about 3:1 can cause lower hybrid
formation. Control reactions utilizing yeast RNA or
globin mRNA can be used, and should show no detectable
30 hybrids, indicating hybridization specificity only to
SV-40 mRNA. Also, thermal denaturation profile studies
and comparison of the kinetics of hybridization can
confirm that the synthetic oligonucleotide reacts only



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with SV-40 mRNA sequences.

5 Once it is shown that the oligonucleotide hybridizes to the isolated SV-40 mRNA, in vitro translation tests can be attempted utilizing the wheat embryo system (described previously) to show that the hybrid is not translated. Basically, upon introduction of SV-40 mRNA into the wheat embryo system, the system produces large T antigen protein. However, when an equal amount or more of synthetic oligonucleotide is also added to the system, T antigen protein synthesis can be substantially inhibited, without interference with synthesis of other SV-40 proteins whose mRNA was also introduced.

10 Testing of the oligonucleotide in vivo can be accomplished by adding the oligonucleotide to cultures of cells infected with SV-40. Synthesis of T antigen protein should be inhibited significantly in about six hours, and SV-40 growth should be strongly inhibited within about 24 hours. The growth of control cultures should be largely unaffected.

20 The synthetic oligonucleotide of the present invention may be mass produced according to common cloning techniques, such as those developed in the art to clone the gene for proinsulin. Alternatively, the oligonucleotide can be chemically synthesized in commercially available equipment (described previously). Briefly, the cloning method entails enzymatic insertion of the oligonucleotide into a bacterial gene carried on a larger piece of DNA, known as a plasmid. The plasmid can be incorporated into a suitable host bacteria, and multiple copies made as the bacteria multiply as in Boyer and Cohen, U. S. Patent No. 4,237,224.



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More particularly, and with reference to FIGS. 4 and 5, the cloning plasmid designated as pBR322, available from Bethesda Research Labs, Inc., Rockville, Maryland, can be used to mass produce the T protein specific oligonucleotide. Using standard techniques, the oligonucleotide is converted to double stranded form and then a terminal 5'PO₄ is added to each of the 5' termini with polynucleotide kinase to permit subsequent joining through T-4 ligase. The reaction conditions for forming the 5' termini can be found in Richardson, Progress in Nucleic Acids Research 2:815 (1972).

After purification of the double stranded oligonucleotide by chromatography on hydroxylapatite columns, it is inserted into the plasmid. Because the oligonucleotide is blunt ended, the plasmid should not have uneven or "sticky" ends. To remove sticky ends from the plasmid, S1 nuclease or other single strand specific nucleases can be utilized. A general description of methods for using restriction nucleases can be found in Nathans and Smith, Annual Review of Biochemistry 44: 273 (1975).

For best results, a linker system between the oligonucleotide and the plasmid can be utilized, specifically a linker having both Hind III and Alu I enzymatic cleaving sites. As seen in FIG. 4, one such linker has a sequence: 5'...CTAAGCTTAG...3'. This sequence represents a double stranded, bisymmetric molecule containing a recognition sequence both for Alu I (AGCT) and for Hind III (AAGCTT). Utilizing DNA ligase under standard conditions, this molecule can be ligated to the oligonucleotide to form linker-oligonucleotide-linker molecules. Similarly, the linked oligonucleotide can be



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intr oduced into linearized blunt-ended, Hind III cl av d
pBR322 carrier molecules.

After ligation, the plasmid has resumed its
covalently closed circular configuration with the linker-
oligonucleotide incorporated, all of which is shown in
FIG. 5 as pT-protein oligonucleotide. The recircularized
plasmid is then used to transform a suitable bacterial
host such as E. coli. The methods for transformation and
selection of transformants are known in the art and
described in detail in Cohen and Boyer, U.S. Patent No.
4,237,224.

Once the transformed bacteria containing the
ligated plasmid p-oligonucleotide have been grown to high
density and produced large amounts of the ligated plas-
mid, the oligonucleotide is ready for purification.
After the plasmid has been removed from the mature cells,
the plasmid is treated with appropriate restriction
endonucleases. As illustrated in FIG. 5, the plasmid is
first cleaved with Hind III to give various by-products,
including linker-T-protein-oligonucleotide-linker
and fragments of the original plasmid. These are readily
separated utilizing gel electrophoresis or high pressure
liquid chromatography. Further cleavage of the isolated
linker-oligonucleotide-linker with the endonuclease Alu I
yields pure double stranded oligonucleotide and partially
degraded linker. These can also be separated based on
their size differences.

As shown in FIG. 6, the oligonucleotide can
then be modified to a nuclease resistant phosphotriester
form utilizing the reaction described in Miller et al,
Biochemistry 16:1988 (1977). Basically, the oligonucleo-



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5 tide is first acylated using 50% acetic anhydride-pyridine during an overnight incubation period. The product is precipitated and isolated from ether. The phosphotriester can then be formed utilizing 30% ethanol in anhydrous 2,6-lutidine (30%), NN-dimethyl formamide (30%) and p-toluene sulfonyl chloride (17%), and reacting for about 6 hours. The protecting acetyl groups are then hydrolyzed by the addition of 0.5 volumes of concentrated ammonium hydroxide, followed by incubation for about 1
10 hour at 55°C. The final oligonucleotide product in the ethyl phosphotriester form can then be isolated on paper chromatography or high pressure liquid chromatography.

15 It is believed that transforming the oligonucleotide to a phosphotriester form will improve the oligonucleotide's stability in vivo due to an enhanced resistance against various degradative enzymes. However, the oligonucleotide will eventually degrade because of spontaneous de-ethylation, which leaves the molecule unprotected. Indeed, by controlling the initial level
20 of ethylation, the in vivo degradation rate can be controlled. A further advantage of a phosphotriester form is believed to be an increase in the oligonucleotide's ability to penetrate a cell membrane.

EXAMPLE 2

25 A synthetic oligonucleotide capable of inhibiting the synthesis of follicle stimulating hormone (FSH), a protein hormone produced by the pituitary that functions in the maturation of ova in females and sperm cells in males, can also be constructed. It is known that FSH
30 is composed of two chains, alpha and beta, the amino acid sequence of which has been determined for several animal



spe i s. Interestingly, th alpha chain of FSH is common to ther g nadotropic h rm nes, including thyr id stimu-
lating h rm ne, luteinizing hormone, and chorionic gonadotropin, while the beta chain varies. Therefore, t
5 selectively shut off the synthesis of FSH without sub-
stantially affecting the other gonadotropins, the oligo-
nucleotide must be specific for the mRNA coding for the
beta chain.

The sequence of the beta chain amino acids 32
10 through 40 is shown in FIG. 7. As discussed earlier, it
is possible to predict the mRNA base sequence for these
amino acids, although not with absolute certainty. Th
points of uncertainty are indicated by the letter "X" in
the predicted mRNA sequence. Thus, the resultant oligo-
15 nucleotide family consists of eight possible 26 base
sequences; the potential alternate bases are shown
in parentheses below the primary base sequence.

By beginning with the projected mRNA sequence
for the 33rd through 40th amino acids, it can be seen
20 that four different 23 base oligonucleotides exist that
could correspond to the FSH mRNA. The sequences could b
as follows, reading from the 5' end: GTGTAGCAGTAGCCGCC-
GCACCA, GTGTAGCAGTATCCGGCGCACCA, GTGTAGCAGTAGCCTGCGCACCA,
and GTGTAGCAGTATCCTGCGCACCA.

25 One of these four sequences should be precisely
correct and thus able to hybridize fully with the FSH
mRNA. To determine the best sequence, a hybridization
test against FSH mRNA, with subsequent purification on
hydroxylapatite or other suitable column, can be per-
30 formed as previously described. Once the best sequence
has been determined, it is placed in a plasmid or



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chemically synthesized, as described above, for bulk synthesis. This oligonucleotide should substantially inhibit the synthesis of FSH in vivo.

5 From the foregoing, it will be appreciated that the present invention provides a systematic method of designing new therapeutic agents for use in living organisms and that this method is versatile and inexpensive. Further, the oligonucleotide produced in accordance with the present invention is extremely effective
10 and specific, enabling selective control of protein synthesis in a living organism.

While several particular forms of the invention have been illustrated and described, it will be apparent that various modifications can be made without departing
15 from the spirit and scope of the invention. Accordingly, it is not intended that the invention be limited, except as by the appended claims.



CLAIMS

1. A method of developing therapeutic agents comprising the steps of: providing a base sequence of an organism's nucleic acid, said base sequence containing at least a portion of genetic information for a biological component of said organism; and synthesizing an oligonucleotide, the sequence of which is derived from said base sequence, for hybridization with messenger ribonucleic acid specific for said biological component.
2. The method of Claim 1 further including the step of: transforming said oligonucleotide into a more stable form to inhibit degradation by said organism.
3. The method of Claim 2 wherein said more stable form is a phosphotriester form.
4. The method of Claim 1 wherein said biological component is a protein.
5. The method of Claim 4 wherein said oligonucleotide is a deoxyribonucleotide.
6. The method of Claim 1 wherein the base sequence comprises about fourteen or more bases.
7. The method of Claim 1 wherein the base sequence comprises about twenty-three bases.
8. The method of Claim 1 wherein the order of said base sequence is determined from ribonucleic acid or deoxyribonucleic acid specific for said biological component prior to synthesizing the oligonucleotide.
9. The method of Claim 1 further comprising the step of: inserting said oligonucleotide into a plasmid for cloning.
10. The method of Claim 9 wherein said oligonucleotide is inserted into said plasmid with a linker base sequence.
11. The method of Claim 10 wherein said linker sequence is GATTCGAATC or CTAAGCTTAG.
12. The method of Claim 10 wherein the linker is susceptible to partial degradation by Hind III or Alu I restriction nuclease.
13. The method of Claim 1 further comprising the



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step f: cross-hybridizing the base sequence against nucleic acid from at least one source other than said organism, whereby the base sequence is more specific to said organism.

14. A method of selectively controlling activity of one or more specific biological components in a cell without substantially interfering with the activity of other biological components of said cell, said method comprising the steps of: forming an oligonucleotide having a base sequence substantially complementary to a portion of messenger ribonucleic acid coding for said biological component; and introducing said oligonucleotide into said cell.

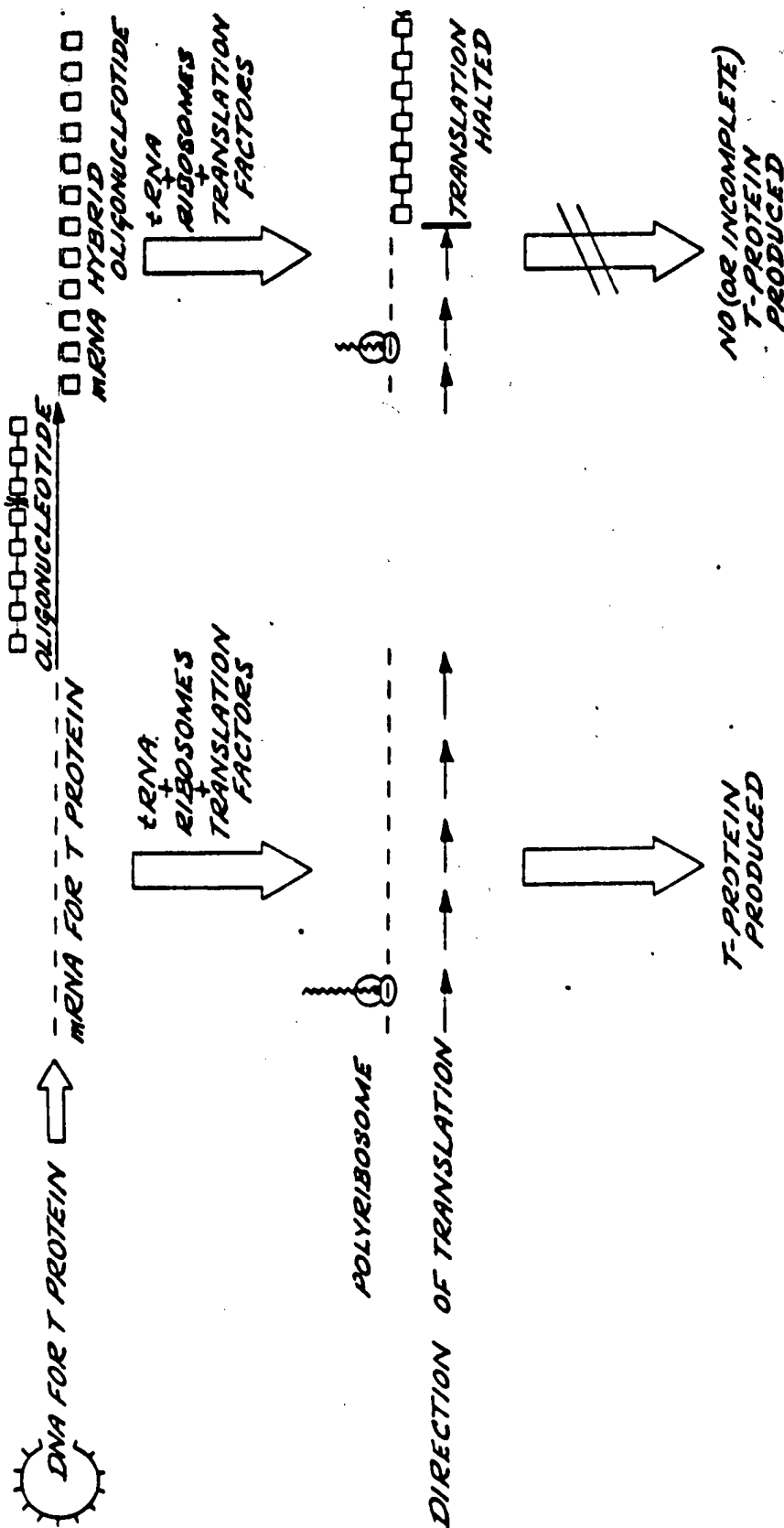
15. A method of inhibiting the infection of a host organism by a foreign organism, said method comprising the steps of: isolating a base sequence from said foreign organism's nucleic acid, the base sequence containing at least a portion of genetic information coding for a protein vital to said foreign organism's viability; synthesizing an oligonucleotide, the order of which is derived from said base sequence to be substantially complementary to messenger ribonucleic acid coding for the protein; and treating said foreign organism with an effective amount of oligonucleotide to hybridize with a portion of said messenger ribonucleic acid and block translation of said protein, thereby inhibiting the viability of the foreign organism.

16. An agent for use in controlling synthesis of a protein, said agent comprising an oligonucleotide in a stabilized form to inhibit degradation and having a nucleotide sequence substantially complementary to a base sequence of at least a portion of messenger ribonucleic acid coding for said protein.





FIG. 2



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FIG.3

OLIGONUCLEOTIDE SPECIFIC FOR SV-40 T PROTEIN

VIRAL DNA(CODING) 3'...G A A C G T T T C T A C C T A T T T C...5'

VIRAL T PROTEIN

mRNA 5'...C U U U G C A A A G A U G G A U A A A G...3'

T PROTEIN SPECIFIC

OLIGONUCLEOTIDE 3'...G A A C G T T T C T A C C T A T T T C...5'

FIG.7

OLIGONUCLEOTIDE SPECIFIC FOR FOLLICLE STIMULATING HORMONE

PROTEIN SEQUENCE:

N TERMINUS...THR TRP CYS ALA GLY TYR CYS TYR THR...C TERMINUS

mRNA PREDICTED:

5' END ACX UGG UGC GCX GGX UAC UGU UAC ACX 3' END

FSH

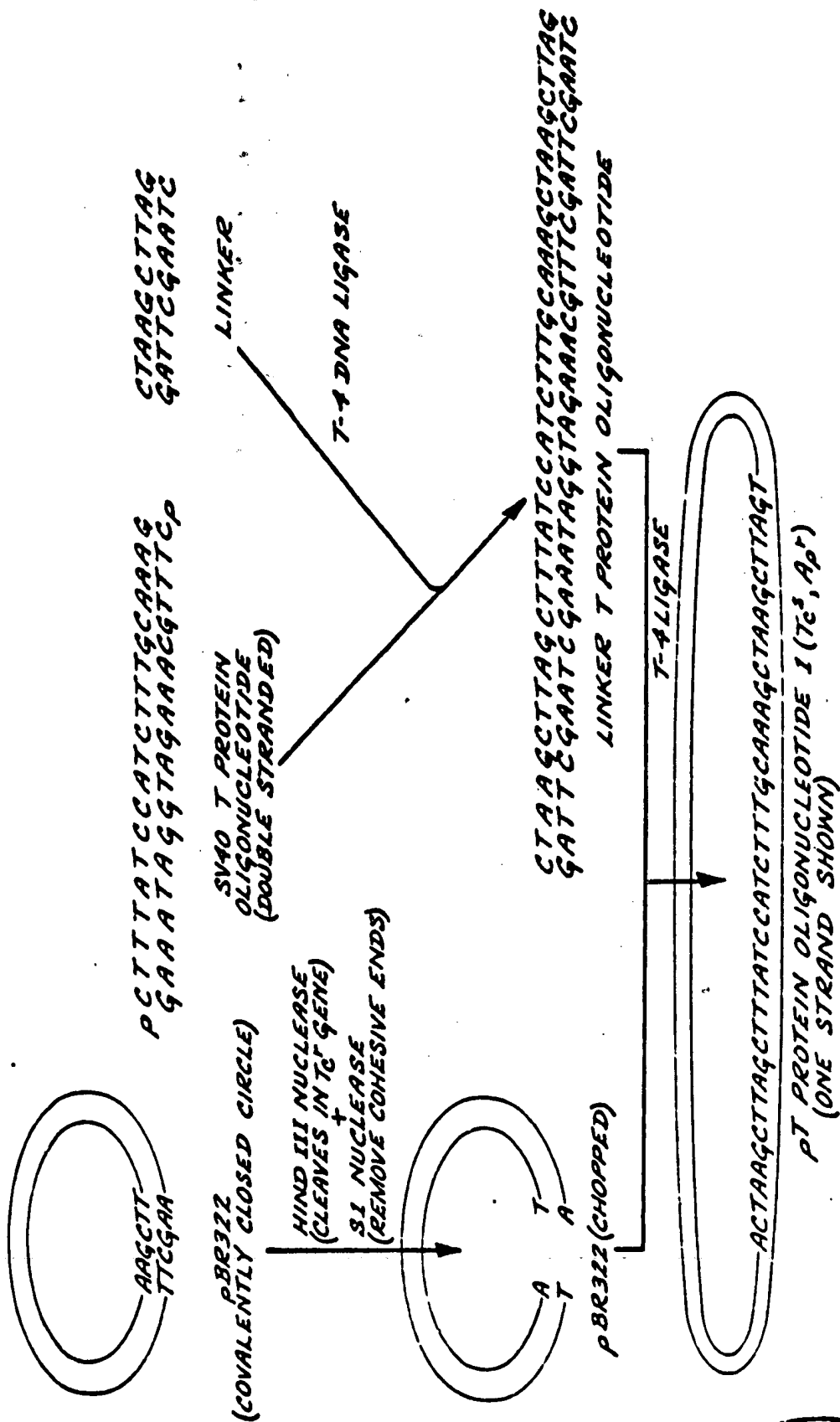
OLIGONUCLEOTIDE FAMILY:

5' END TCG ACC ACC CCG CCG ATG ACG ATG TG- 5' END
(T) (T) (T)

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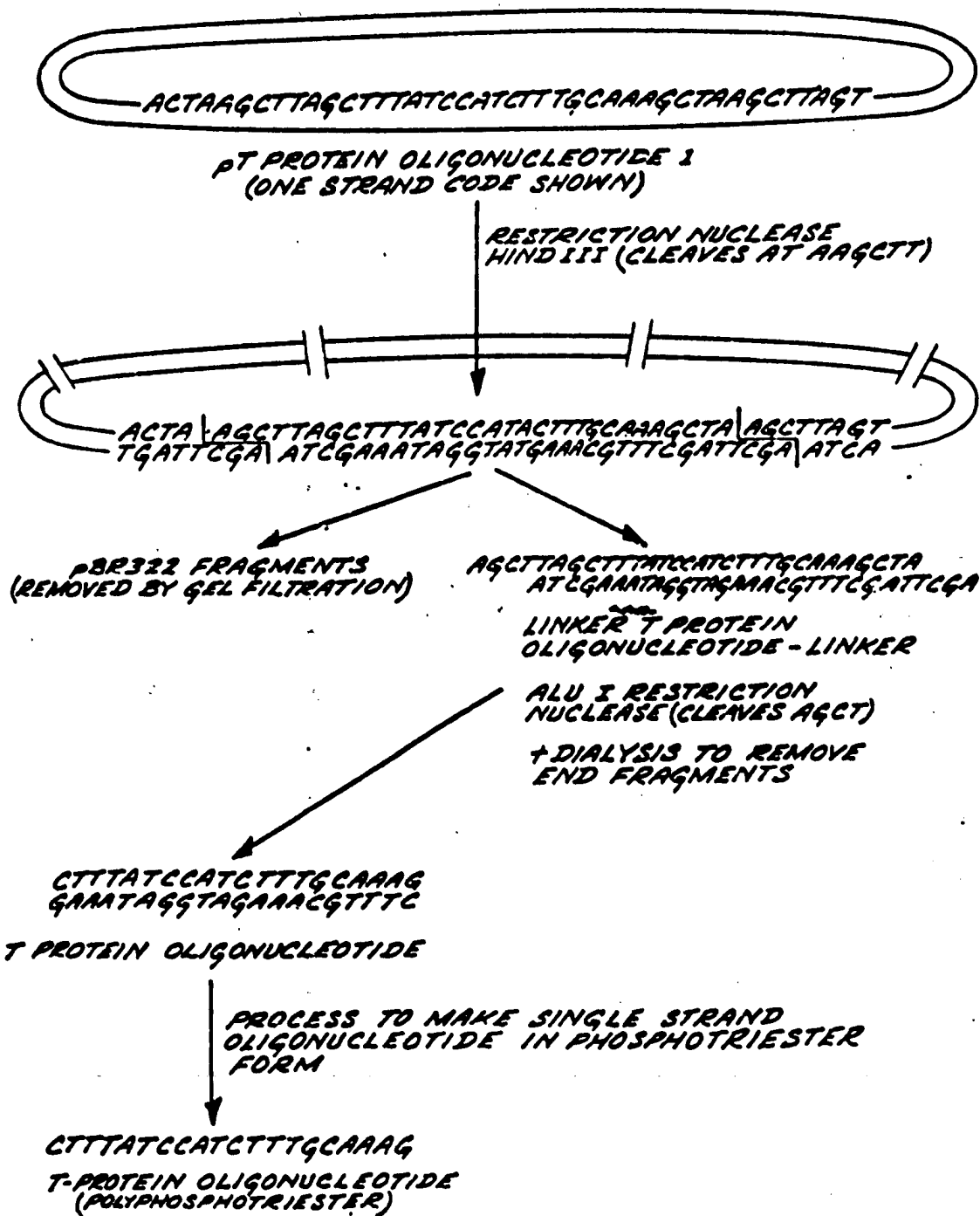
FIG. 4
CONSTRUCTION OF HIGH YIELD PLASMID CONTAINING THE T PROTEIN OLIGONUCLEOTIDE



415

FIG. 5

PRODUCTION OF SV-40 T PROTEIN OLIGONUCLEOTIDE FROM
PT OLIGONUCLEOTIDE USING RESTRICTION NUCLEASE
CLEAVAGE TO RELEASE FRAGMENTS.

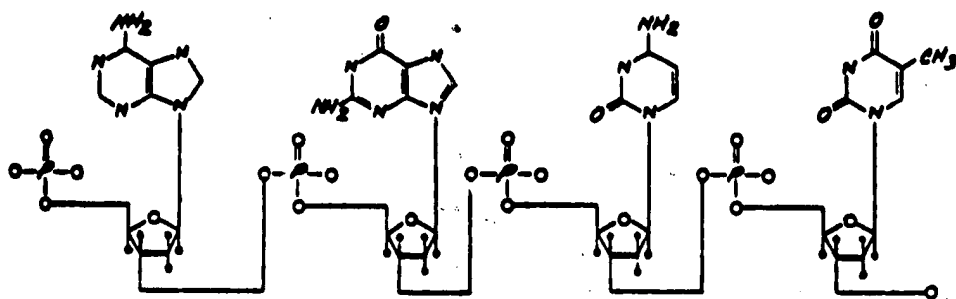


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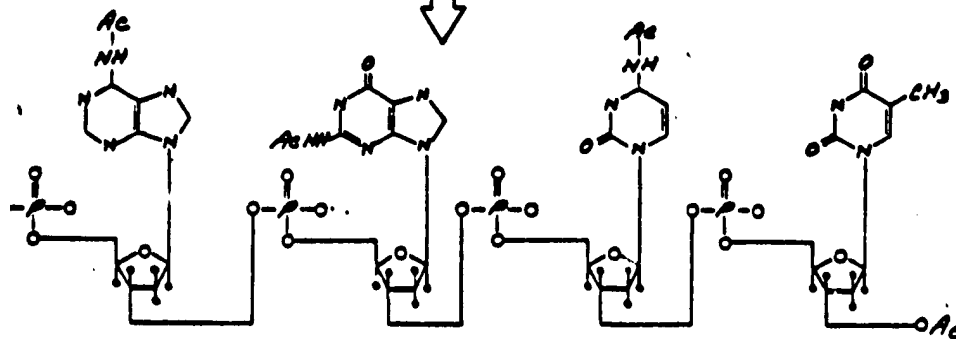
FIG 6



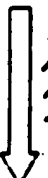
DNA SEGMENT



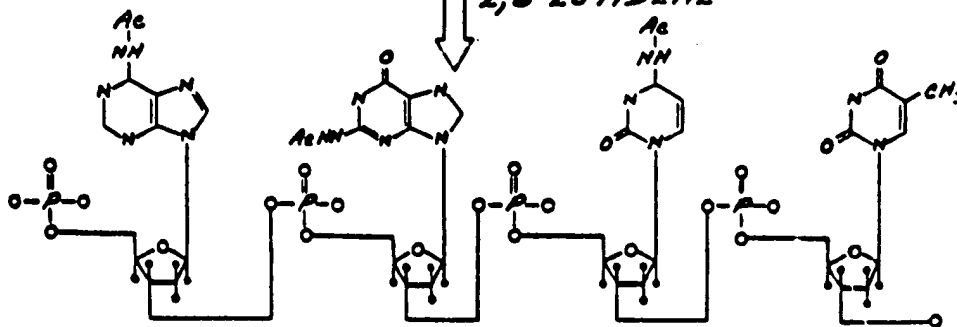
ACETIC ANHYDRIDE



ACETYLATED DNA (BASE PROTECTED)



30% ETHANOL
 NN-DIMETHYLFORMAMIDE
 17% TOLUENE SULFONYL CHLORIDE
 2,6 LUTIDENE



DNA POLYPHOSPHOTRIESTER (BASE PROTECTED)



DNA POLYPHOSPHOTRIESTER

SUBSTITUTE SHEET



L. CLASSIFICATION OF SUBJECT MATTER (If several classifications are used, give them in order of importance)

According to International Patent Classification (IPC) or to other national Classification: 37 and 38

IPC: C07H 21/02, C07H 21/04, C12N 15/00, C12Q 1/68, C12P 21/00, C12P 19/34

M. FIELDS SEARCHED

Classification System

Classification System

U.S.

424/200; 435/6,68,70,91,172,317; 536/27

Documentation Searched other than Minimum Documentation:
to the extent that such documents are included in the Fields Searched

MEDLINE 1966-82

BIOSIS PREVIEWS 1969-83

LIFE SCIENCES COLLECTION 1978-82

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No. 1
A	US, A, 4,237,224, PUBLISHED 02 DECEMBER 1980, COHEN ET AL.	1-16
A,P	US, A, 4,321,365, PUBLISHED 23 MARCH 1982, WU ET AL.	1-12, 16
A	GB, A, 2,068,971A, PUBLISHED 19 AUGUST 1981, CAREY ET AL.	1-16
A	N, WALLACE ET AL, NUCLEIC ACIDS RESEARCH, VOLUME 6, NUMBER 11, 1979, PAGES 3543-57.	1-16
X	N, INGLIS ET AL, VIROLOGY, VOLUME 78, 1977, PAGES 522-36.	1,4,6,7,14-16
A	N, HASTIE ET AL, PROC. NATL. ACAD. SCI., USA, VOLUME 75, NUMBER 3, MARCH 1978, PAGES 1217-21.	1-16
A	N, ALVARADO-URBINA ET AL, SCIENCE, VOLUME 214, 16 OCTOBER 1981, PAGES 270-74.	1-12, 16
X	N, PATERSON ET AL, PROC. NATL. ACAD. SCI., USA, VOLUME 74, NUMBER 10, OCTOBER 1977, PAGES 4370-74.	1,4,5-7,9,14,16
X	N, ITAKURA ET AL, SCIENCE, VOLUME 209, 19 SEPTEMBER 1980, PAGES 1401-1405.	1-12, 16

* Special categories of cited documents: 13

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is considered with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

29 DECEMBER 1982

Date of Mailing of this International Search Report

03 JAN 1983

International Searching Authority

ISA/US

Signature of Authorizing Officer

JAMES MARTINELL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	N, PATERSON ET AL, PROC. NATL. ACAD. SCI., USA, VOLUME 74 NUMBER 10, OCTOBER 1977, PAGES 4370-74.	2,3,8,10-13 13
Y	N, INGLIS ET AL, VIROLOGY, VOLUME 78, 1977, PAGES 522-26.	2,3,5,8-13

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSearchable ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

☐ Claim numbers _____, because they relate to subject matter not required to be searched by this Authority, namely:

☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹⁰, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS Lacking ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claim numbers:

☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.